On-Job Training Report:
Wet Lab Practices in a Professional Scientific Laboratory at National Facility for Biopharmaceuticals, Matunga, Mumbai

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Guru Nanak Khalsa College
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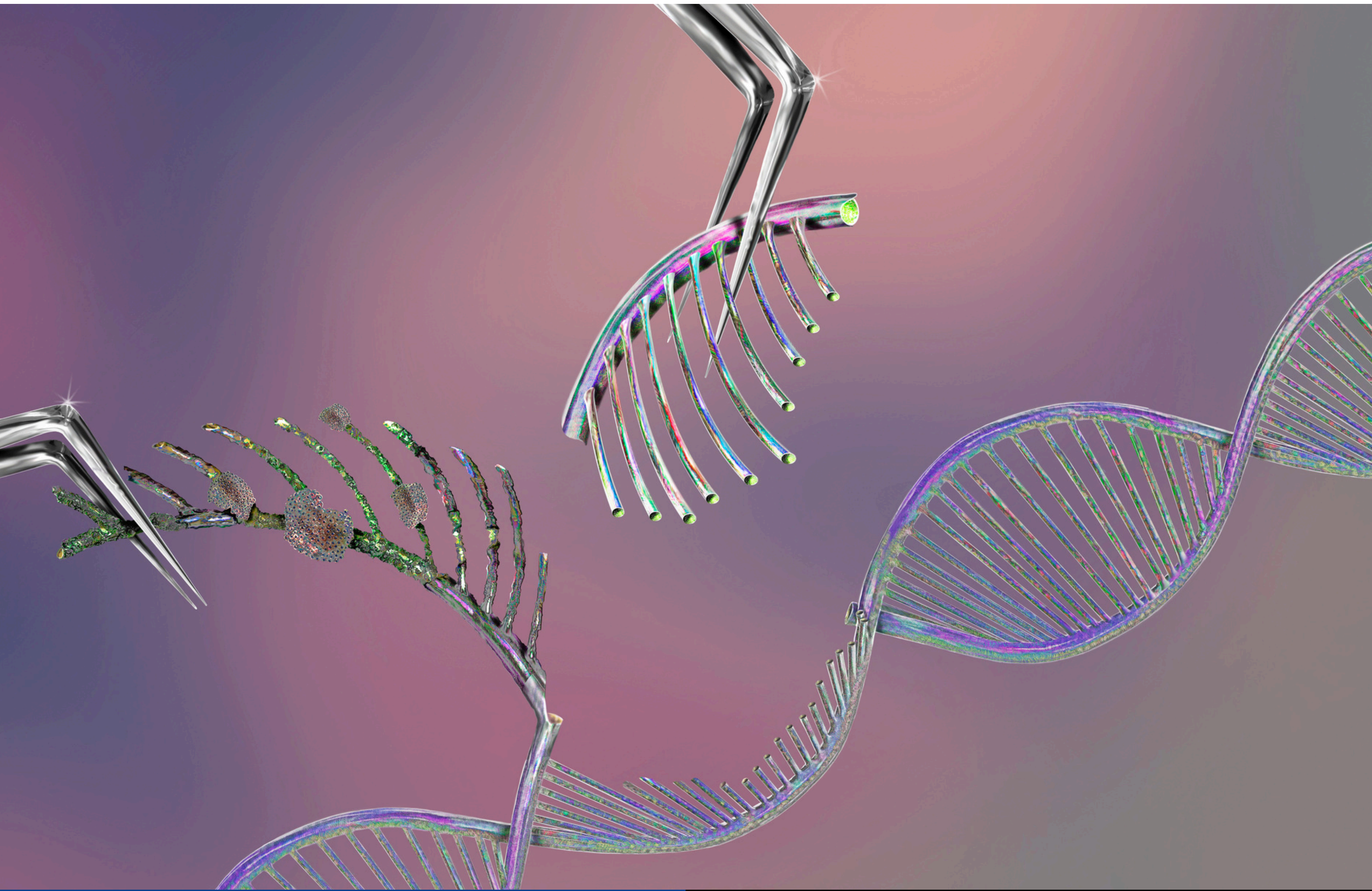
NFB
Transforming Education into Expertise



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We gratefully acknowledge the expertise of Ms. Valencia D'Souza, Senior Research Fellow, in molecular biology, particularly Real-time PCR. Her training and demonstrations have been invaluable for our skill development. We also appreciate the contributions of Mr. Jaydeep, Ms. Sakshi Padawe, and Ms. Ira Kode, whose dedicated support has ensured the smooth functioning of various facility activities, enhancing our learning experience.

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Date: 27/04/2024

On-Job Training Report: Wet Lab Practices in a Professional Scientific Laboratory at National Facility for Biopharmaceuticals, Matunga, Mumbai

Introduction to Molecular Biology

Molecular biology stands as a cornerstone of modern biological research, offering a profound understanding of life at the fundamental level of molecules. This discipline meticulously dissects the intricate interplay between various biomolecules, such as DNA, RNA, and proteins, within the cellular milieu. By elucidating these molecular mechanisms, researchers gain invaluable insights into the core biochemical processes that govern all aspects of biological function, encompassing growth, reproduction, metabolism, and cellular response to stimuli.

The term "molecular biology" itself emerged in 1938, by the courtesy of American scientist Warren Weaver. As advancements in physics and chemistry yielded powerful tools like X-ray diffraction and electron microscopy, the field blossomed, enabling biologists to delve deeper into the captivating world of biomolecules. While observations of microscopic structures within living organisms date back to the 18th century, a comprehensive understanding of their molecular basis truly materialized in the 20th century.

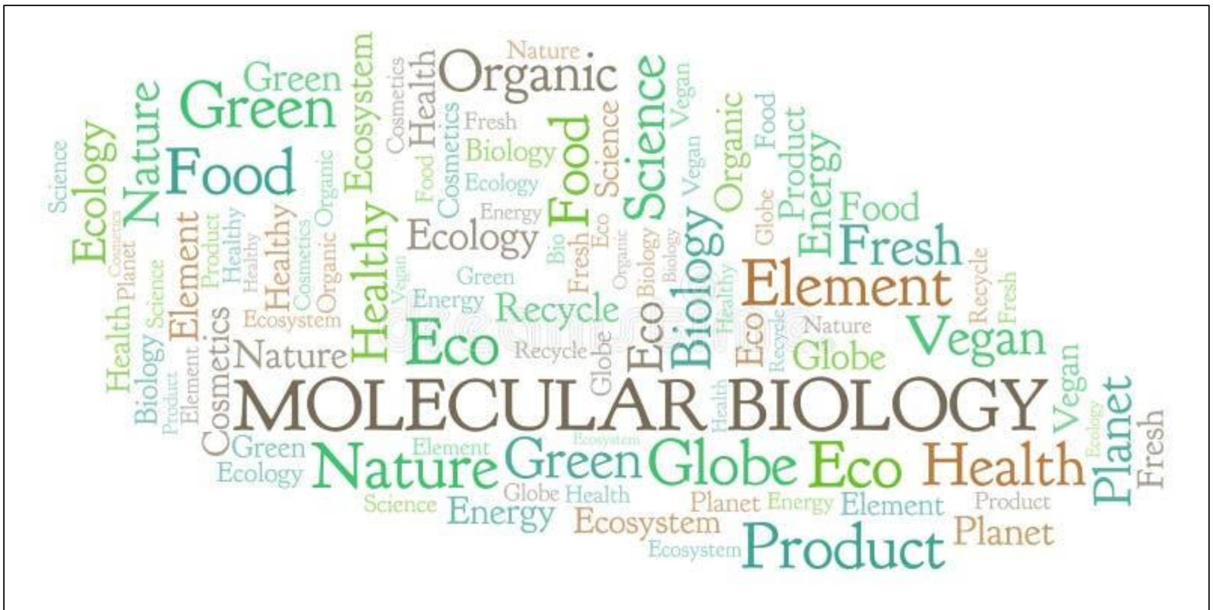

Figure 1: Word cloud of Molecular biology

The Ever-Expanding Landscape of Molecular Biology

The future of molecular biology is brimming with possibilities that extend far beyond our current understanding. Fueled by continuous advancements in technology and our ever-deepening grasp of molecular mechanisms, researchers are on the verge of unlocking groundbreaking discoveries that have the potential to reshape healthcare, agriculture, and our very perception of biology. Some prominent avenues of exploration include:

1. **Gene Therapy:** This revolutionary technique holds immense promise for tackling a wide range of diseases at their very root by directly modifying genes that cause or contribute to the disease. For example, gene therapy has the potential to cure cystic fibrosis, a genetic disorder that affects the lungs and digestive system, by introducing a healthy copy of the defective gene into lung cells. Another area of active research is in the use of gene therapy to treat cancer. By introducing genes that suppress tumor growth or enhance the immune system's ability to recognize and destroy cancer cells, gene therapy offers a promising approach for cancer treatment.

2. **Personalized Medicine:** By leveraging an individual's unique genetic makeup, personalized medicine aims to tailor treatments and preventative measures for optimal efficacy. This approach takes into account an individual's genes, environment, and lifestyle to predict their risk of developing certain diseases and to identify the most effective treatment strategies. For example, in the case of cancer, personalized medicine can involve analyzing a patient's tumor to identify specific genetic mutations that are driving the cancer's growth. This information can then be used to select targeted therapies that are more likely to be effective for that particular patient.

3. **Synthetic Biology:** This burgeoning field paves the way for the engineering of novel biological systems with functionalities not found in nature. Synthetic biologists draw inspiration from natural biological systems but use their understanding of molecular mechanisms to design and construct new systems with unique properties. These engineered systems have the potential for a wide range of applications, including the development of new biofuels, the production of novel drugs, and the creation of biosensors for environmental monitoring.

4. **Deciphering Complex Diseases:** Unveiling the intricate interplay of molecules that contribute to the pathogenesis of complex diseases like cancer and Alzheimer's is crucial for developing effective interventions. By studying the molecular pathways involved in these diseases, researchers can identify potential targets for drug development. Additionally, a deeper understanding of the molecular basis of complex diseases can lead to the development of new diagnostic tools and preventative strategies.

The Wet Lab: The Engine Room of Discovery

Wet labs are the engine room of discovery because they bring theories to life. Scientists can directly manipulate biomolecules, validate ideas, and identify limitations in models. Powerful tools like microscopes and genetic engineering allow for observation and interaction with biomolecules, providing deeper understanding. Additionally, wet labs embrace the unexpected. Unforeseen results can spark new research avenues and challenge existing beliefs. Finally, the iterative nature of wet lab research is key. Experiments can lead to further investigation and refinement, fostering the scientific method's core principle of continuous improvement. This hands-on, adaptable, and discovery-driven environment makes the wet lab the heart of scientific progress in molecular biology.

Figure 2: Molecular Biology Laboratory

The wet lab serves as the physical manifestation of the scientific method in molecular biology. This specialized laboratory is meticulously equipped with sophisticated instruments and tools designed for the manipulation and analysis of biomolecules. Here are some of the cornerstones of wet lab research:

1. **Gel electrophoresis:** A cornerstone technique for separating biomolecules based on their size and charge. An electric current is passed through a gel matrix, with smaller molecules migrating faster through the pores of the gel. By analyzing the position of bands on the gel

after staining, researchers can identify and estimate the size of biomolecules within a sample. This technique is widely used for separating DNA fragments, proteins, and RNA molecules.

2. **Polymerase Chain Reaction (PCR):** A powerful tool for amplifying specific DNA sequences. PCR utilizes a heat-resistant DNA polymerase enzyme, along with primers (short DNA sequences complementary to the target region) and nucleotides, to exponentially replicate a targeted DNA segment. This technique has revolutionized molecular biology, enabled the analysis of minute DNA samples and facilitated various downstream applications, such as DNA cloning and sequencing.

3. **DNA sequencing:** This process determines the precise order of nucleotides (adenine, guanine, cytosine, and thymine) within a DNA molecule. Understanding the DNA sequence is essential for deciphering the genetic makeup of an organism, identifying mutations associated with diseases, and unraveling the function of genes. Various DNA sequencing technologies have emerged, each with its own advantages and limitations. Newer techniques offer high-throughput sequencing, enabling the rapid analysis of vast amounts of DNA data.

4. **Protein purification:** Isolating specific proteins from a complex mixture is a prerequisite for studying their structure and function in detail. Wet lab researchers employ a multitude of techniques for protein purification, including chromatography, precipitation, and centrifugation. These techniques exploit the unique physical and chemical properties of proteins to separate them from other cellular components.

The wet lab is the beating heart of molecular biology research. Here, scientists meticulously manipulate and analyze biomolecules, transforming theoretical concepts into tangible discoveries. This realm serves as the critical bridge between the elegance of molecular design and the magnificent tapestry of life itself.

By fostering a deeper understanding of the molecular underpinnings of life, molecular biology paves the way for advancements in healthcare, agriculture, and our fundamental comprehension of the biological world. As this field continues to evolve, it holds the potential to revolutionize countless aspects of human experience.

Computational Biology

Computational biology is a dynamic interdisciplinary field at the intersection of biology, computer science, mathematics, and statistics. It leverages computational methods to analyze biological systems across different scales, from molecular interactions to organismal behavior. This field encompasses diverse research areas, including genomics, proteomics, systems biology, evolutionary biology, and bioinformatics. Through high-throughput experimental techniques like DNA sequencing and mass spectrometry, vast amounts of biological data are generated, which computational biologists then analyze using a range of computational tools and algorithms. Integration of experimental data with computational approaches, such as sequence alignment, phylogenetic analysis, protein structure prediction, and network modeling, allows researchers to uncover patterns and principles underlying biological processes.

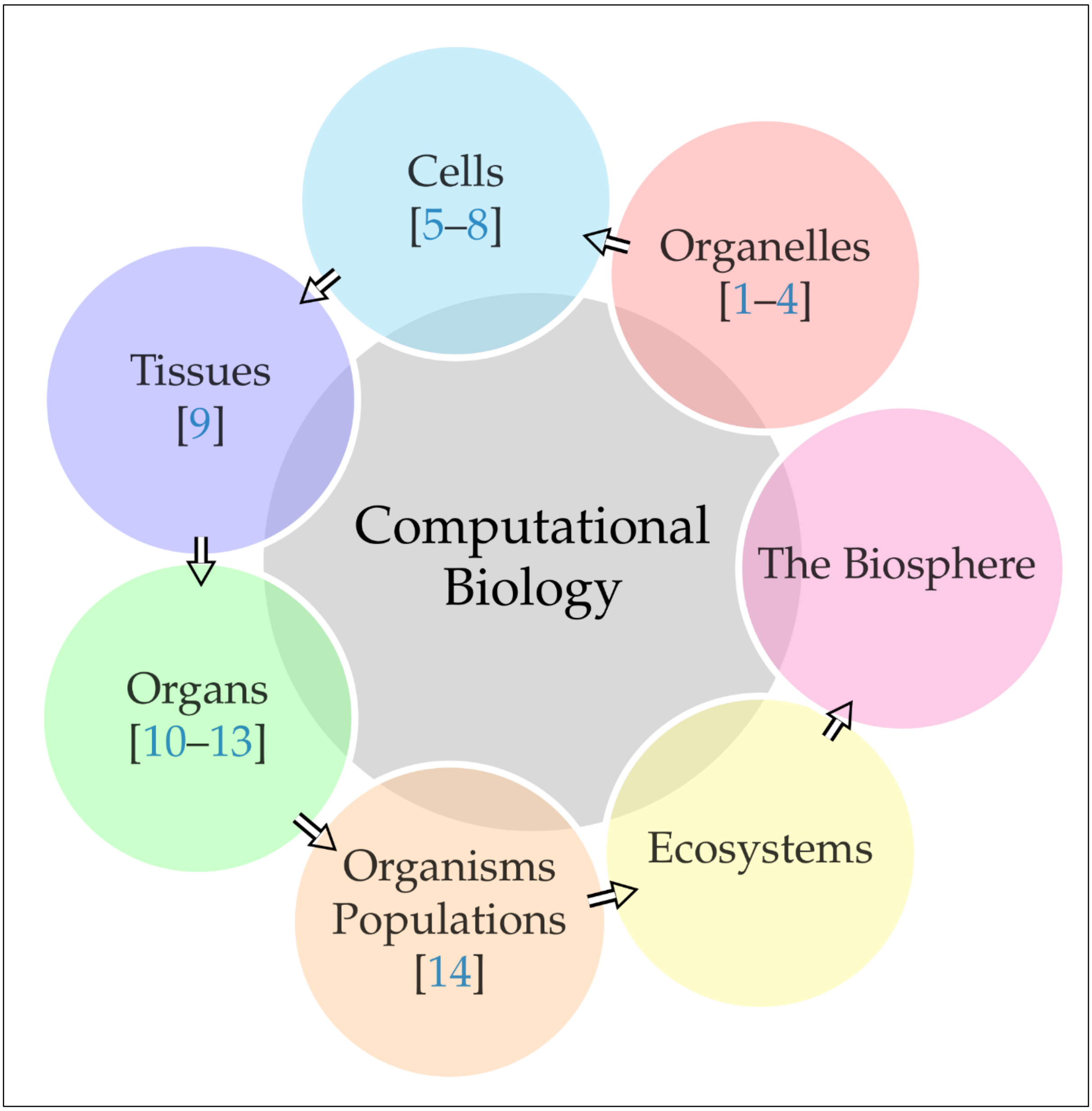

Figure 3: Areas that can be studies using Computational Biology

The impact of computational biology extends across various domains, including medicine, biotechnology, and environmental science. It has revolutionized disease gene identification, protein structure prediction, drug development, and our understanding of evolutionary and ecological dynamics. With the exponential growth of biological data and advancements in high-performance computing and machine learning, computational biology continues to evolve rapidly. These developments enhance the capabilities of computational biologists to tackle increasingly complex biological problems, paving the way for new scientific discoveries and insights. Overall, computational biology represents a dynamic and transformative field that has reshaped our approach to biological research, offering novel opportunities for exploration and innovation.

Bioinformatics

Bioinformatics is an interdisciplinary field that combines biology, computer science, and information technology to manage, analyze, and interpret biological data. It involves the development and application of computational tools and algorithms to address a wide range of biological problems, from genomics and proteomics to systems biology and evolutionary studies.

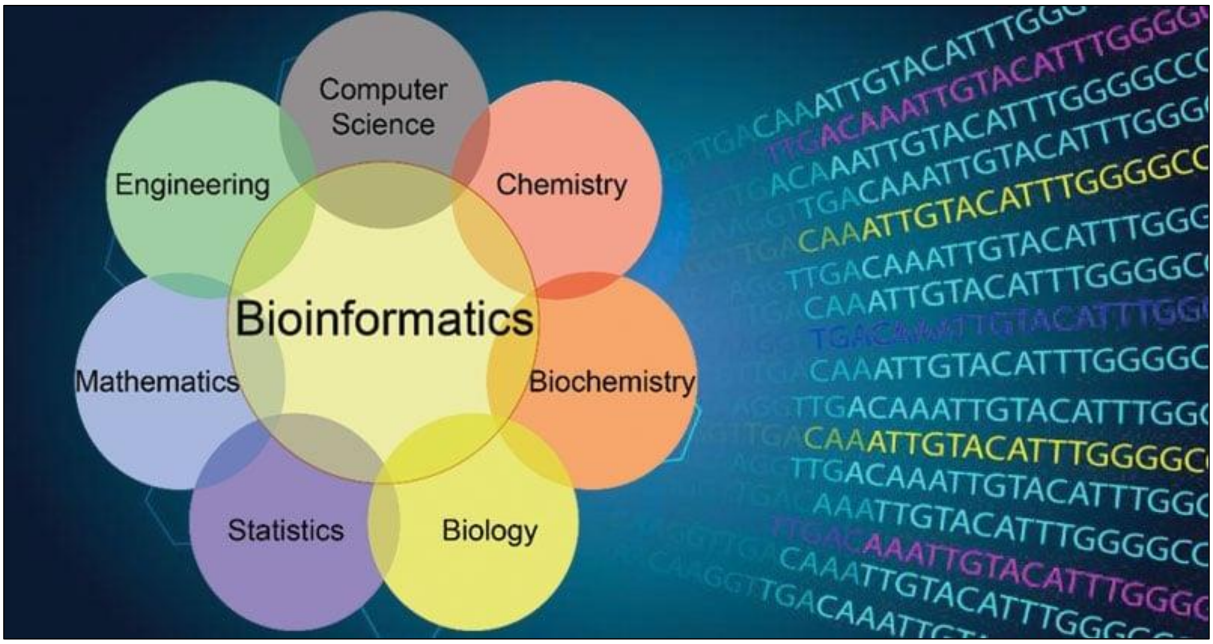

Figure 4: Fields coinciding with Bioinformatics

The primary goals of bioinformatics include:

1. **Data Management:** Bioinformaticians develop and maintain databases and repositories to store, organize, and provide access to large amounts of biological data, such as DNA sequences, protein structures, and gene expression profiles.
2. **Data Analysis:** Bioinformaticians design and implement computational algorithms and software tools to analyze and interpret biological data, enabling the identification of patterns, relationships, and insights that may not be readily apparent from raw data.
3. **Biological Modeling:** Bioinformaticians develop computational models and simulations to represent and study biological systems, from the molecular level to the organismal level, to gain a deeper understanding of their structure, function, and dynamics.
4. **Biological Discovery:** Bioinformatics plays a crucial role in the discovery of new genes, proteins, and other biological entities, as well as the identification of potential drug targets and the development of new therapeutic approaches.

Bioinformatics relies on a wide range of computational techniques, including sequence alignment, phylogenetic analysis, structural modeling, machine learning, and data visualization. These methods are applied to various biological data sources, such as genomic sequences, protein structures, gene expression profiles, and metabolic pathways.

The field of bioinformatics has experienced rapid growth and has become an essential component of modern biological research. Bioinformaticians collaborate closely with experimental biologists, providing computational support and insights that drive the advancement of our understanding of biological systems. As the volume and complexity of biological data continue to increase, the role of bioinformatics in facilitating scientific discoveries and translating them into practical applications is expected to become even more crucial.

In Silico Experimentation

In silico experimentation, also known as computational experimentation, refers to the use of computer simulations and models to study biological systems and processes. This approach complements traditional in vivo (in living organisms) and in vitro (in a controlled laboratory environment) experiments, providing a powerful tool for investigating complex biological phenomena.

In silico experimentation involves the development of computational models and simulations that represent various aspects of biological systems, such as molecular interactions, cellular pathways, tissue dynamics, or population-level behaviors. These models are based on experimental data, theoretical knowledge, and computational algorithms, and they allow researchers to explore and test hypotheses in a virtual environment.

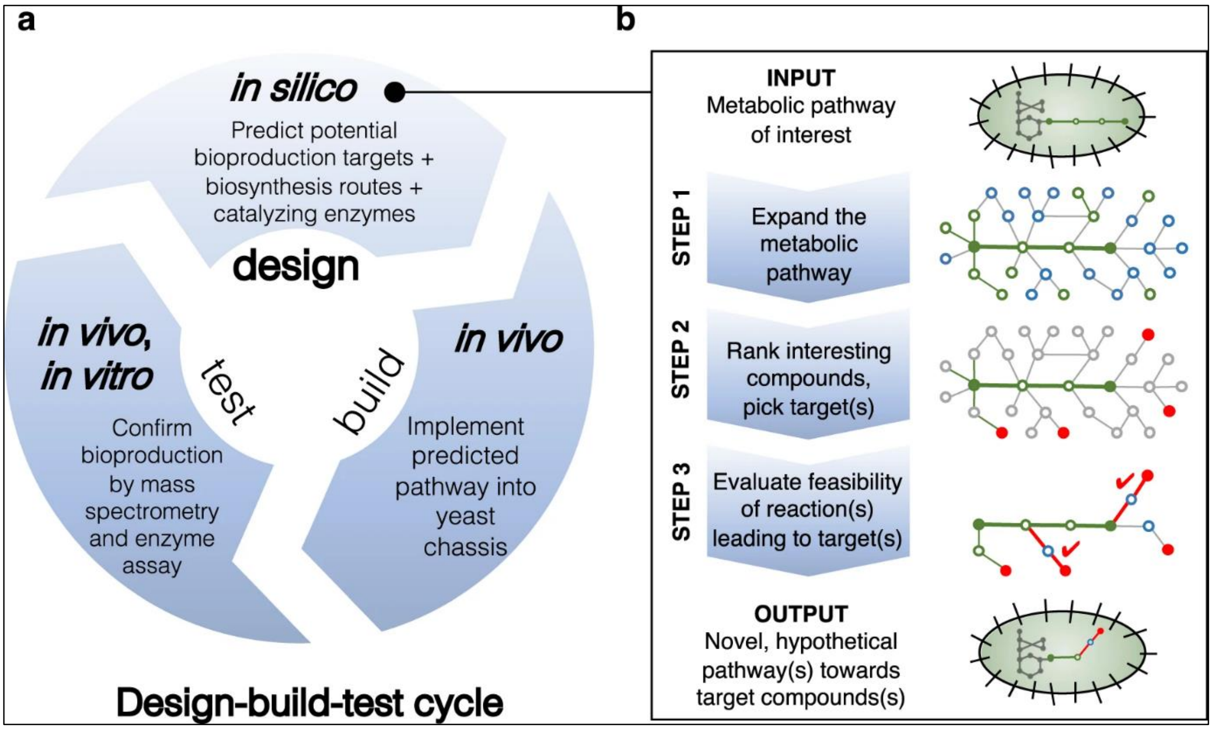

Figure 5: In silico experimentation workflow

The key advantages of in silico experimentation include:

1. **Efficiency and Cost-effectiveness:** In silico experiments can be conducted more rapidly and at a lower cost compared to traditional wet-lab experiments, as they do not require the same resources and infrastructure.
2. **Scalability and Complexity:** Computational models can handle large-scale, complex biological systems that would be challenging or impossible to study experimentally.
3. **Hypothesis Testing:** In silico experiments enable researchers to test multiple hypotheses and scenarios quickly, guiding the design of subsequent wet-lab experiments.
4. **Ethical Considerations:** In silico approaches can reduce the need for animal testing and experiments that may raise ethical concerns.

In silico experimentation has been widely adopted in various fields of biology, including drug discovery, systems biology, evolutionary biology, and ecology. Researchers use computational tools and techniques, such as molecular dynamics simulations, agent-based modeling, and network analysis, to gain insights into biological processes and make predictions that can be validated through experimental studies.

The integration of in silico experimentation with traditional wet-lab approaches has become a powerful strategy in modern biological research, allowing for a more comprehensive understanding of complex biological systems and accelerating the pace of scientific discovery.

Molecular Biology Methods and Techniques Supporting In-Silico Experimentation & Bioinformatics Analysis

Experimental molecular biology techniques such as DNA sequencing, gene expression analysis, protein purification, and structural biology serve as the cornerstone for in silico experimentation in computational biology and bioinformatics. These techniques yield vast amounts of data that enable the construction of accurate computational models of biological processes. Computational biologists leverage this data to simulate and predict complex biological phenomena, facilitating a deeper understanding of molecular-level interactions. The emergence of computational biology and bioinformatics has been driven by the need to analyze and interpret the massive datasets generated by experimental molecular biology. Through the integration of computational methods and experimental data, researchers can uncover hidden patterns and relationships within biological systems, advancing our understanding of complex biological processes and driving innovation in the field.

In silico experimentation offers a powerful means of studying biological systems, particularly those challenging to investigate experimentally. By employing computational models and simulations, researchers can explore diverse hypotheses and scenarios in a virtual environment, providing valuable insights that guide experimental design and interpretation. This approach allows scientists to overcome experimental limitations and simulate biological processes without the need for extensive empirical data. The integration of experimental molecular biology with computational technology is vital for refining in silico experimentation techniques, facilitating deeper insights into biological complexities, and fostering innovation within the realm of molecular biology.

Molecular biology methods and techniques act as the backbone for in silico experimentation and bolster the acceptance of computational biology results among bioinformaticians in several ways:

1. **Validation and Ground Truthing:** In silico predictions, generated through computer simulations and modeling, require real-world validation to establish their accuracy and reliability. Wet lab experiments in molecular biology provide this essential validation by allowing researchers to test the predictions made by computational models. For instance, computational modeling might predict how a particular drug molecule interacts with a protein target. Wet lab experiments can then be conducted to confirm these predictions, using techniques like protein-protein interaction assays. This validation process strengthens confidence in the in silico results and paves the way for their wider application.
2. **Data Generation and Refinement:** In silico analyses rely heavily on robust and comprehensive biological data. Molecular biology techniques play a crucial role in generating this data, such as DNA sequencing for gene identification, protein purification for structural analysis, and gene expression studies to understand cellular processes. This data serves as the fuel for computational models, allowing researchers to refine their algorithms and improve the accuracy of their predictions. For example, protein structures determined through X-ray crystallography or cryo-electron microscopy in wet labs can be

used to develop and refine computational models for protein folding and protein-protein interactions.

3. **Hypothesis Generation and Experimental Design:** The insights gleaned from in silico analyses can inspire the formulation of novel hypotheses in molecular biology. Computational models can identify potential drug targets, predict protein function, or suggest regulatory pathways within cells. These predictions can then be translated into testable hypotheses in the wet lab. Furthermore, in silico modeling can aid in the design of wet lab experiments by optimizing reaction conditions, identifying essential controls, and predicting potential outcomes. This synergy between computational and wet lab approaches allows researchers to delve deeper into biological questions and accelerate scientific discovery.
4. **Bridging the Gap Between Bench and Bioinformatics:** Molecular biology techniques bridge the gap between the wet lab bench and the world of bioinformatics. By providing a tangible context for computational models, wet lab experiments enhance the understanding and appreciation of in silico methods among bioinformaticians. Conversely, researchers in molecular biology gain valuable insights from computational predictions, allowing them to design more targeted and efficient experiments. This fosters a collaborative environment where both disciplines work in tandem to unlock the secrets of life.

In essence, molecular biology methods and techniques serve as the crucial link between theory and practice in computational biology. They validate predictions, generate data, inspire hypotheses, and bridge the gap between scientific disciplines. This synergy strengthens the foundation of in silico experimentation and increases the acceptance of computational biology results within the bioinformatics community.

Purpose of the Hands-on Training in Molecular Biology

Despite the burgeoning field of bioinformatics, bioinformaticians face a unique set of challenges in the job market, such as:

1. **The Interdisciplinary Gap:** Bioinformatics sits at the intersection of biology and computer science. Employers often seek candidates who possess a strong foundation in both disciplines. This can be particularly challenging for individuals with a strong background in one area but limited experience in the other.
2. **Rapidly Evolving Field:** The field of bioinformatics is constantly evolving with advancements in technology and the emergence of new computational tools. This necessitates continuous learning and adaptation for bioinformaticians to stay relevant in the job market.
3. **Data Deluge:** The exponential growth of biological data presents both opportunities and challenges. Bioinformaticians need the skills to effectively manage, analyze, and interpret vast datasets, which requires not only computational expertise but also an understanding of the biological context behind the data.
4. **Communication Divide:** Bridging the communication gap between bioinformaticians and wet lab researchers is crucial for successful project execution. Bioinformaticians need to be able to translate complex computational concepts into clear and understandable terms for biologists, while also grasping the biological questions and challenges faced by their wet lab counterparts.

Equipping bioinformaticians with hands-on training in molecular biology techniques can empower them to overcome these challenges and thrive in the job market as it can help in various ways such as:

1. **Bridging the Interdisciplinary Gap:** Practical experience in the wet lab fosters a deeper comprehension of biological processes. This allows bioinformaticians to design more relevant computational analyses and interpret their results within a meaningful biological context.
2. **Understanding the Data:** Hands-on experience with molecular biology techniques provides bioinformaticians with a firsthand understanding of how biological data is generated. This knowledge empowers them to effectively evaluate data quality, identify potential biases, and choose appropriate computational tools for analysis.
3. **Collaboration and Communication:** Working in a wet lab environment fosters collaboration and communication with wet lab researchers. Bioinformaticians gain valuable insights into the challenges faced by their counterparts, enabling them to communicate complex computational concepts more effectively.
4. **Translating Theory to Practice:** Wet lab training allows bioinformaticians to test and validate their computational predictions in real-world scenarios. This practical experience strengthens their understanding of the limitations and strengths of computational methods, leading to more robust and reliable analyses.

In conclusion, hands-on training in molecular biology equips bioinformaticians with a well-rounded skillset that bridges the gap between computation and biology. This comprehensive understanding enhances their ability to tackle complex biological questions, collaborate effectively with researchers, and ultimately, thrive in the dynamic bioinformatics job market.

Why National Facility for Biopharmaceuticals (NFB)?

The National Facility for Biopharmaceuticals (NFB) stands out as a premier destination for hands-on Molecular Biology training in India. It offers specialized modules covering Molecular Biology, Protein Purification, Fermentation, and Mammalian tissue culture techniques. With a comprehensive curriculum tailored to industry needs, NFB ensures students receive thorough training in specific job functions and regulatory requirements within the Biopharma sector. The training programs focus on developing research aptitude and soft skills, preparing students for seamless integration into the industry. Backed by experienced faculty, NFB provides students with expert guidance and mentorship. Its accessibility, welcoming visits from affiliated colleges every Saturday, further enhances its appeal. Emphasizing practical learning, NFB's modules comprise 80% hands-on training, offering students a well-rounded educational experience. In essence, NFB's specialized curriculum, industry-oriented approach, experienced faculty, and accessibility make it an outstanding choice for aspiring professionals seeking hands-on Molecular Biology training.

Figure 6: National Facility for Biopharmaceuticals, Matunga

The National Facility for Biopharmaceuticals (NFB) is an excellent choice for hands-on Molecular Biology training for the following reasons:

1. **Specialized Training:** NFB is the first of its kind facility in India that is committed to providing "Hands-On" training on various aspects of the Biopharma industry, with a

specialization in Molecular Biology, Protein Purification, Fermentation, and Mammalian tissue culture techniques.

2. **Comprehensive Curriculum:** NFB offers a comprehensive offering of hands-on training modules that cover the many aspects of specific job functions, regulations, and applications found within the Biopharma industry.
3. **Industry-Oriented Approach:** The training programs at NFB are designed to develop a research aptitude and enhance the soft skills of the students, making them industry-ready.
4. **Experienced Faculty:** NFB has compiled a team of qualified teachers and mentors who can provide students with the necessary guidance and support to succeed in the Biopharma industry.
5. **Accessibility:** NFB is open for visits by students and teachers from all affiliated colleges of the University of Mumbai on every Saturday between 2 pm to 6 pm, making it easily accessible.
6. **Hands-On Training:** The training modules at NFB involve 80% practical and 20% theory, ensuring that students get a comprehensive, hands-on learning experience.

In summary, the National Facility for Biopharmaceuticals (NFB) is an excellent choice for hands-on Molecular Biology training due to its specialized curriculum, industry-oriented approach, experienced faculty, and accessibility to students and teachers.

Trainers/Teachers:

The National Facility for Biopharmaceuticals (NFB) boasts a team of exceptional professionals who are leaders in their respective fields. Mr. Vikas Jha, Head of Molecular Biology Lab, is renowned for his dedication to teaching and mentorship, instilling a passion for molecular biology in his students. Mr. Dinesh Kumar, Head of Proteomics & Protein Characterization Lab, drives innovation and excellence in proteomics research through his expertise and visionary leadership. Ms. Valencia D'Souza, Senior Research Fellow, illuminates the world of molecular biology with her depth of understanding in Real-time PCR, providing invaluable hands-on training and contributing to cutting-edge research endeavors. Together, these individuals exemplify the commitment to excellence and advancement of knowledge that defines the ethos of NFB.

Figure 7: Team National Facility for Biopharmaceuticals

1. **Mr. Vikas Jha (Head of Molecular Biology Lab)**
   Mr. Vikas Jha, the Head of the Molecular Biology Lab at the National Facility for Biopharmaceuticals (NFB), is a highly esteemed trainer and mentor in the field of molecular biology. With his wealth of expertise and passion for the subject, Vikas has become a revered figure among students and professionals alike. His dynamic teaching style and ability to break down complex concepts into understandable pieces elevate the learning experience for all. Beyond the classroom, Vikas fosters a supportive environment where students feel empowered to explore and grow. Under his guidance, the Molecular Biology Lab thrives as a center of innovation, equipped with state-of-the-art resources. Vikas's dedication to education and commitment to student success ensure that the lab remains at the forefront of molecular biology education, inspiring the next generation of scientists to push the boundaries of discovery.

2. **Mr. Dinesh Kumar (Head of Proteomics & Protein Characterization Lab)**
   Mr. Dinesh Kumar, as the Head of the Proteomics & Protein Characterization Lab at the National Facility for Biopharmaceuticals (NFB), brings extensive expertise and leadership

to his role. His profound understanding of proteomics and protein characterization drives innovation and excellence in research within the lab. Under his guidance, the lab remains at the forefront of technological advancements, leveraging cutting-edge equipment and methodologies to unravel the complexities of protein structure and function. Mr. Kumar fosters a collaborative environment that promotes interdisciplinary research and engages with academia, industry, and the broader scientific community to drive impactful change in the field of proteomics. Overall, Mr. Dinesh Kumar's leadership is characterized by his dedication to advancing research, empowering researchers to push the boundaries of scientific knowledge in the dynamic field of protein science.

3. **Ms. Valencia D'Souza (Senior Research Fellow)**
   Ms. Valencia D'Souza, a Senior Research Fellow at the National Facility for Biopharmaceuticals (NFB), is a distinguished expert in molecular biology, particularly in Real-time PCR (polymerase chain reaction). Her profound understanding of this technique allows her to provide comprehensive hands-on training and insightful demonstrations to students and professionals. Valencia's expertise in Real-time PCR enables her to craft engaging lessons that unravel its complexities, making it accessible to learners of all levels. She seamlessly integrates theory with practical applications, empowering participants to gain both theoretical knowledge and practical skills essential for success in the field. Beyond her teaching role, Valencia actively contributes to research projects at NFB, leveraging her expertise to advance scientific knowledge in various areas of molecular biology. In summary, Ms. Valencia D'Souza's role as a Senior Research Fellow and expert in Real-time PCR shines a light on the world of molecular biology at NFB, inspiring the next generation of scientists and contributing to the advancement of knowledge in this dynamic field.

4. **Mr. Jaydeep, Ms. Sakshi Padawe and Ms. Ira Kode (Assistants)**
   Mr. Jaydeep, Ms. Sakshi Padawe, and Ms. Ira Kode serve as valuable assistants at the National Facility for Biopharmaceuticals (NFB), contributing to the smooth functioning of various activities within the facility.
   Mr. Jaydeep plays a crucial role in providing technical & methodical support in Proteomics lab, ensuring that operations run efficiently. His organizational skills and attention to detail help in coordinating schedules, managing resources, and facilitating communication among team members.
   Ms. Sakshi Padawe and Ms. Ira Kode brings their expertise in laboratory management to the team, assisting in the maintenance of equipment and supplies necessary for research activities. Their meticulous approach ensures that the laboratory operates smoothly, enabling experimentations effectively easy.
   Together, Mr. Jaydeep, Ms. Sakshi Padawe, and Ms. Ira Kode play integral roles in supporting the mission of NFB. Their contributions behind the scenes contribute to the success of research projects and educational initiatives, making them valuable members of the team.

Wet Lab Experiments/Methodologies Covered

During the on-the-job training at the National Facility for Biopharmaceuticals (NFB), trainees had ample opportunities to learn from experienced trainers, but the primary emphasis was on acquiring in-depth knowledge and proficiency in the methodologies and techniques integral to molecular biology and proteomic analysis. The training sessions provided comprehensive insights into various molecular biology techniques, including DNA sequencing, gene expression analysis, protein purification, and structural biology, as well as advanced proteomic analysis methods. Participants received hands-on experience in operating state-of-the-art equipment and performing experiments, ensuring they gained practical skills essential for success in the field. Additionally, the trainers imparted theoretical knowledge, elucidated complex concepts, and guided participants through real-world applications, fostering a deeper understanding of the subject matter and preparing them for future endeavors in biopharmaceutical research and development.

1. Isolation of Plasmid DNA (pET21b) from *E. coli* by alkaline lysis method

Plasmid DNA isolation is a fundamental technique in molecular biology and genetic engineering. Plasmids are small, circular DNA molecules that exist independently of the host cell's chromosomal DNA and can be replicated and expressed within the host. The alkaline lysis method is a widely used protocol for the isolation of plasmid DNA from bacterial cells, such as *Escherichia coli* (*E. coli*).

The alkaline lysis method involves several key steps. First, the bacterial cells are lysed using a solution containing sodium hydroxide and sodium dodecyl sulfate (SDS), which disrupts the cell membrane and denatures the cellular proteins. This is followed by the addition of a neutralizing solution, typically potassium acetate, which causes the denatured proteins, chromosomal DNA, and cell debris to precipitate, while the smaller plasmid DNA remains in solution. The plasmid DNA is then separated from the precipitated material by centrifugation, and the supernatant containing the plasmid DNA is collected.

The isolated plasmid DNA can be further purified using various techniques, such as ethanol precipitation, column chromatography, or gel electrophoresis, depending on the intended use of the plasmid. The purity and yield of the plasmid DNA obtained using the alkaline lysis method are generally sufficient for many common applications in molecular biology, such as DNA sequencing, restriction enzyme analysis, and genetic manipulation.

The alkaline lysis method is a simple, efficient, and cost-effective way to isolate plasmid DNA from bacterial cells, making it a widely used technique in both research and diagnostic settings.

Principle:

The principle of isolating plasmid DNA (*pET21b*) from *E. coli* using the alkaline lysis method is based on the selective denaturation and precipitation of cellular components.

The key steps are:

1. Bacterial cells are lysed using a solution containing sodium hydroxide (NaOH) and sodium dodecyl sulfate (SDS). This disrupts the cell membrane and denatures cellular proteins and chromosomal DNA.
2. A neutralizing solution, typically potassium acetate, is added. This causes the denatured proteins, chromosomal DNA, and cell debris to precipitate, while the smaller, circular plasmid DNA remains in solution.
3. The plasmid DNA is separated from the precipitated material by centrifugation. The supernatant containing the plasmid DNA is collected.
4. The plasmid DNA can be further purified using techniques such as ethanol precipitation, column chromatography, or gel electrophoresis, depending on the intended use.
5. The alkaline lysis method effectively separates the plasmid DNA from the host cell's chromosomal DNA and other cellular components, providing a relatively pure and concentrated sample of the plasmid DNA for downstream applications.

Diagrammatical representation:

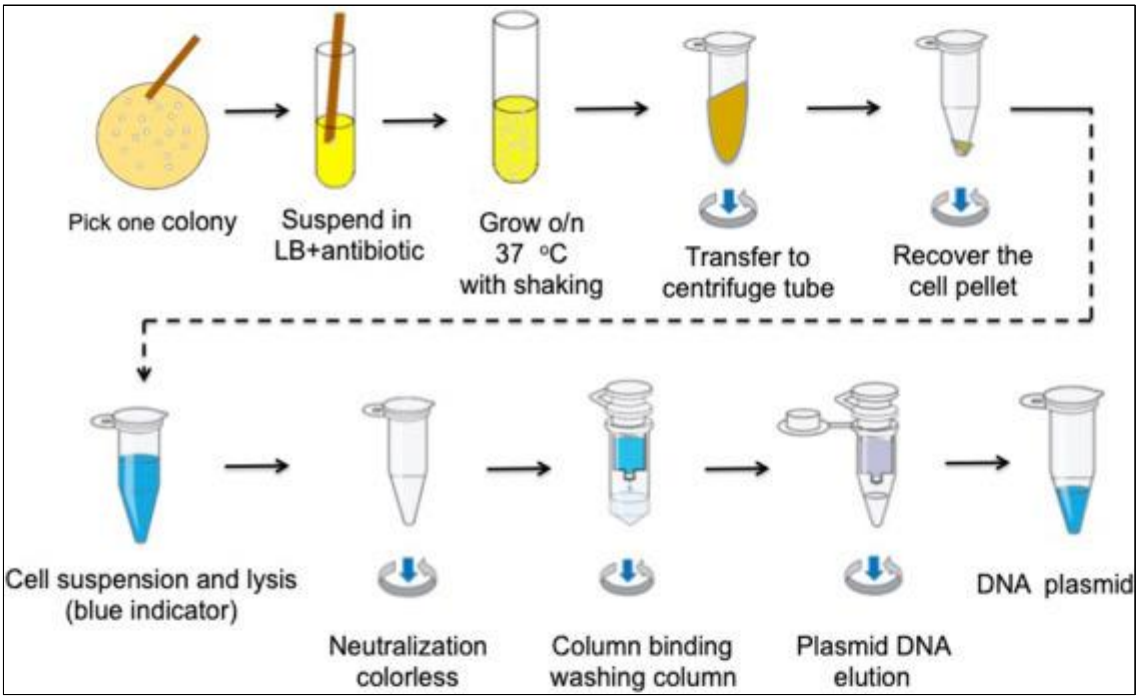

Figure 8: Isolation of Plasmid DNA from *E. coli*

Skills acquired:

Micro-pipetting, Gel preparation & loading, cell lysis and plasmid isolation

2. Isolation of Genomic DNA (gDNA) from *Salmonella typhimurium* by Cetyltrimethylammonium bromide (CTAB) method

The isolation of genomic DNA (gDNA) from *Salmonella typhimurium* is a crucial step in various molecular biology and genomic studies. One widely used method for this purpose is the Cetyltrimethylammonium bromide (CTAB) method. This method is effective in extracting high-quality gDNA from a variety of bacterial species, including *Salmonella Typhimurium*.

The CTAB method involves several key steps. First, a bacterial culture of *Salmonella Typhimurium* is grown overnight in a nutrient-rich medium, such as tryptic soy broth (TSB). The cells are then harvested by centrifugation and the cell pellet is resuspended in a CTAB extraction buffer. This buffer contains the cationic detergent CTAB, which helps to lyse the bacterial cell walls and membranes, releasing the cellular contents, including the gDNA.

Next, the cell lysate is incubated at an elevated temperature, typically around 65°C, to facilitate the denaturation of proteins and the release of the gDNA. Following this, chloroform is added to the mixture, and the solution is gently mixed to extract the gDNA from the cellular debris and other contaminants. The aqueous phase, containing the gDNA, is then separated by centrifugation and transferred to a new tube.

The gDNA is then precipitated by the addition of isopropanol or ethanol, and the resulting pellet is washed with ethanol to remove any remaining impurities. Finally, the purified gDNA is resuspended in a suitable buffer, such as Tris-EDTA (TE) buffer, and is ready for downstream applications, such as sequencing or genomic analysis.

Principle:

The principle of isolating genomic DNA (gDNA) from *Salmonella Typhimurium* using the Cetyltrimethylammonium bromide (CTAB) method is based on the selective precipitation and purification of DNA from cellular components.

The key steps involve:

1. Cell lysis: The CTAB buffer, containing the cationic detergent CTAB, disrupts the bacterial cell walls and membranes, releasing the cellular contents, including the gDNA.
2. Protein denaturation: Incubation at an elevated temperature (typically 65°C) denatures proteins and other cellular components, further facilitating the release of gDNA.
3. Organic extraction: The addition of chloroform allows for the separation of the gDNA-containing aqueous phase from the denatured proteins and other cellular debris.
4. DNA precipitation: The gDNA is then precipitated from the aqueous phase using isopropanol or ethanol, and the resulting pellet is washed to remove any remaining impurities.
5. DNA resuspension: The purified gDNA is finally resuspended in a suitable buffer, such as Tris-EDTA (TE) buffer, for downstream applications.

This method effectively isolates high-quality gDNA from *Salmonella Typhimurium*, which can then be used for various molecular biology and genomic studies.

Diagrammatical representation:

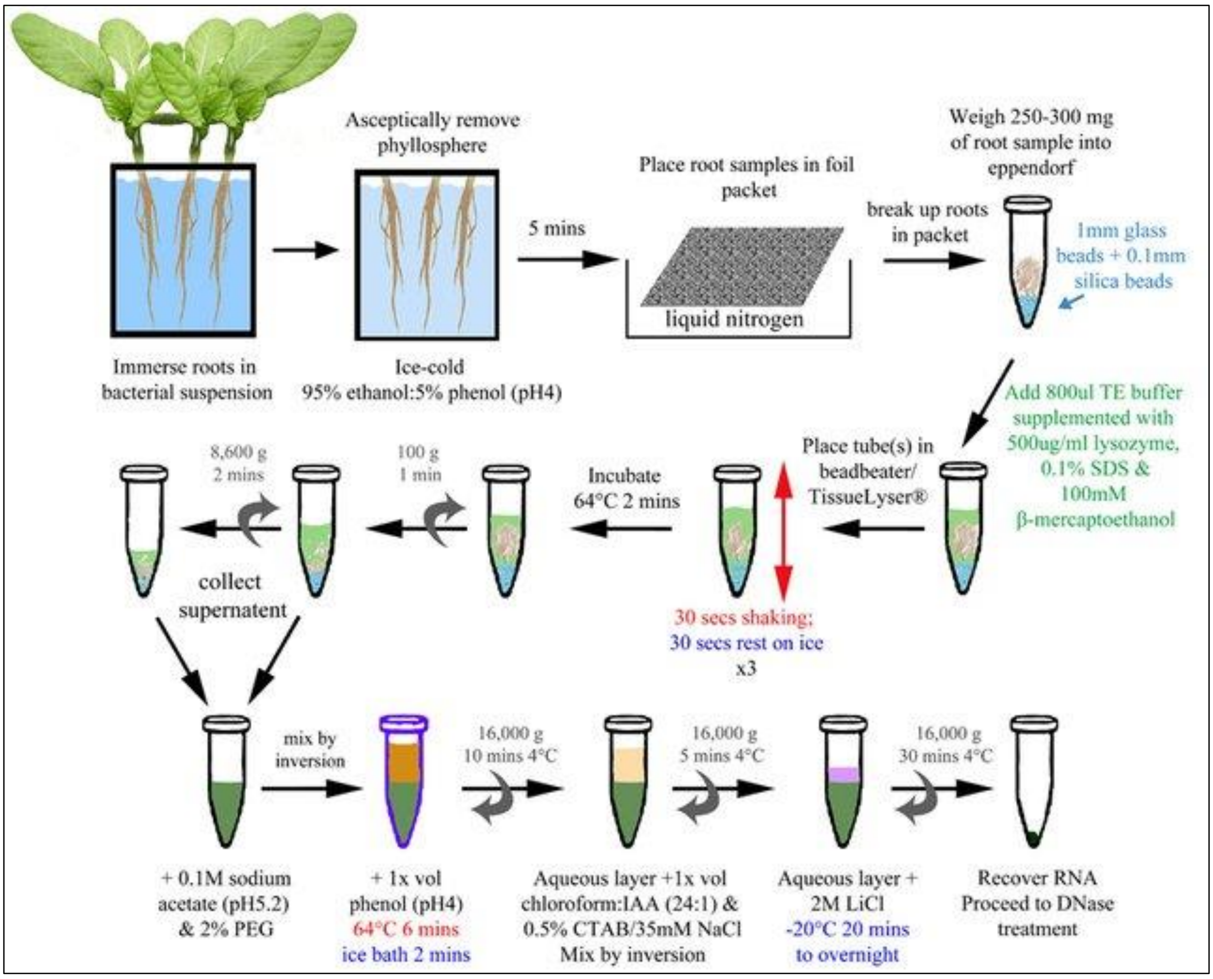

Figure 9: Isolation of Genomic DNA (gDNA) by CTAB method

Skills acquired:

Micro-pipetting, Gel preparation & loading, cell lysis and DNA isolation

3. Restriction enzyme digestion of the isolated plasmid by *Bam*H1 and *Pst*1

Restriction enzyme digestion is a widely used technique in molecular biology and biotechnology to analyze and manipulate DNA sequences. This process involves the use of specific enzymes, as restriction enzymes, which recognize and cleave DNA at specific nucleotide known sequences, known as restriction sites.

The isolated plasmid can be subjected to digestion by the restriction enzymes BamH1 and Pst1 to assess its structure and composition. BamH1 is a type II restriction enzyme that recognizes and cleaves the palindromic DNA sequence 5'-GGATCC-3', while Pst1 recognizes and cleaves the sequence 5'-CTGCAG-3'.

The digestion of the plasmid with these two enzymes can provide valuable information about the plasmid's structure, such as the presence and location of specific DNA sequences, the size of the plasmid, and the presence of any insertions or deletions.

By analyzing the resulting DNA fragments generated by the restriction enzyme digestion, researchers can gain insights into the plasmid's composition and potentially identify any modifications or changes that have been made to the original plasmid sequence. This information can be crucial for various applications, such as gene cloning, protein expression, and the development of recombinant DNA technologies.

Overall, the restriction enzyme digestion of the isolated plasmid by BamH1 and Pst1 is a fundamental technique in molecular biology and biotechnology, providing valuable information about the structure and composition of the plasmid, which can be used to further understand and manipulate the DNA sequences of interest.

Principle:

The principle of restriction enzyme digestion involves the specific cleavage of DNA at particular recognition sites by restriction enzymes. In the case of the isolated plasmid, the enzymes BamH1 and Pst1 are commonly used for this purpose. BamH1 recognizes the sequence "GGATCC" and cleaves between the G and the first A, generating sticky ends with overhangs that are complementary to each other. On the other hand, Pst1 recognizes the sequence "CTGCAG" and cleaves between the C and the first T, also producing sticky ends with complementary overhangs.

When the isolated plasmid is subjected to restriction enzyme digestion with BamH1 and Pst1, these enzymes will specifically bind to their respective recognition sequences on the plasmid and cleave the DNA at those sites. This process results in the generation of linearized DNA fragments with cohesive ends that can be easily ligated with other DNA fragments that have been digested with the same enzymes. This technique is fundamental in molecular biology for creating recombinant DNA molecules, gene cloning, and various genetic engineering applications. The precise cleavage of DNA by restriction enzymes like BamH1 and Pst1 allows for the controlled manipulation and modification of DNA sequences for further analysis and experimentation.

Diagrammatical representation:

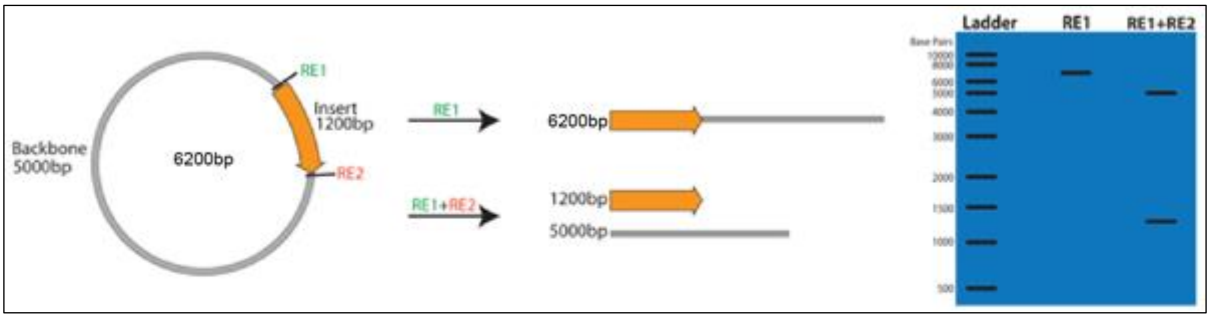

Figure 10: Restriction digestion of plasmid DNA

Skills acquired:

Micro-pipetting, gel loading, DNA cleaving and DNA digestion

4. Ligation of DNA by T4 DNA Ligase

T4 DNA ligase is a crucial enzyme used in molecular biology and genetic engineering to join DNA fragments together. This process, known as DNA ligation, is essential for various applications, including cloning, gene assembly, and DNA repair.

The T4 DNA ligase enzyme catalyzes the formation of a phosphodiester bond between the 3' hydroxyl group of one DNA strand and the 5' phosphate group of another DNA strand. This reaction requires the presence of ATP as a cofactor, which provides the energy necessary for the ligation process.

The ligation reaction mediated by T4 DNA ligase involves three main steps:

1. Activation of the 5' phosphate group: The enzyme first activates the 5' phosphate group of one DNA strand by forming a covalent enzyme-AMP intermediate.
2. Transfer of the AMP group: The activated AMP group is then transferred to the 3' hydroxyl group of the other DNA strand, creating a high-energy phosphoanhydride bond.
3. Formation of the phosphodiester bond: Finally, the enzyme catalyzes the formation of a stable phosphodiester bond between the two DNA strands, completing the ligation process.

T4 DNA ligase is widely used in various molecular biology techniques, such as the construction of recombinant DNA molecules, the joining of DNA fragments during cloning, and the repair of DNA breaks. Its ability to ligate both cohesive (sticky) and blunt-ended DNA fragments makes it a versatile tool in the field of genetic engineering.

Principle:

DNA ligation is a fundamental technique in molecular biology that involves the joining of DNA fragments using an enzyme called T4 DNA ligase. This enzyme catalyzes the formation of a phosphodiester bond between the 3' hydroxyl group of one DNA fragment and the 5' phosphate group of another, effectively sealing the gap between them.

The ligation of DNA fragments is crucial for various applications, such as gene cloning, the construction of recombinant DNA molecules, and the assembly of larger DNA sequences from smaller fragments. T4 DNA ligase is commonly used for this purpose as it can efficiently ligate both cohesive (sticky) and blunt-ended DNA fragments.

The ligation reaction typically involves the incubation of the DNA fragments with T4 DNA ligase, ATP, and a suitable buffer. The enzyme recognizes the complementary ends of the DNA fragments and catalyzes the formation of the phosphodiester bond, effectively joining the fragments together.

The efficiency of the ligation reaction can be influenced by various factors, such as the concentration of the DNA fragments, the ratio of the fragments, the incubation time, and the temperature. Optimizing these parameters is crucial for achieving successful ligation and the subsequent cloning or assembly of the desired DNA constructs.

Diagrammatical representation:

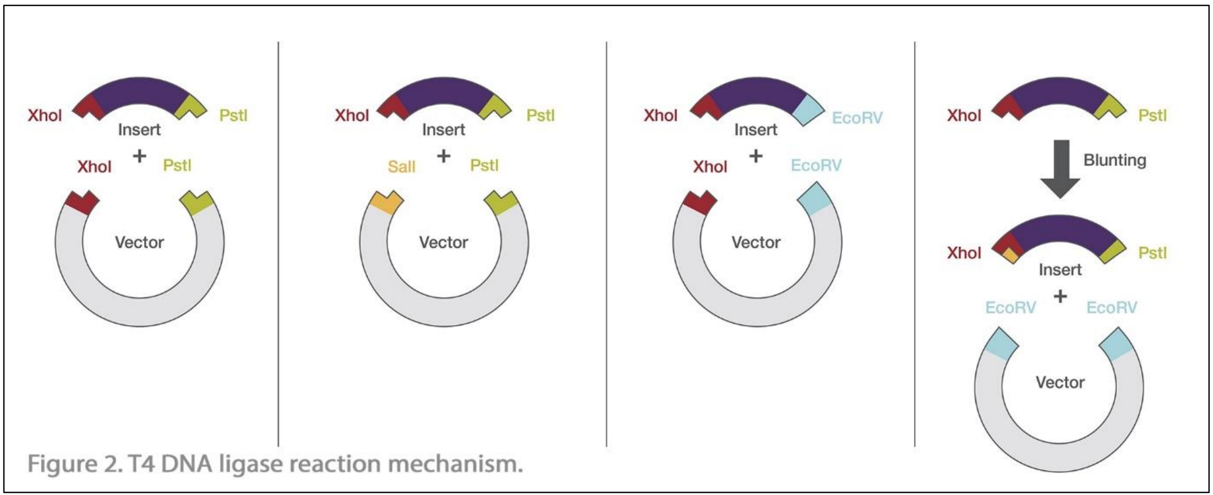

Figure 11: Ligation of DNA by T4 DNA Ligase

Skills acquired:

Micro-pipetting, gel loading, DNA Ligation

5. <u>Separation of Protein by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)</u>

Proteins are fundamental molecules in biological systems, playing crucial roles in various cellular functions. Separating proteins based on their molecular weight is essential for studying their structure and function. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a widely used technique in biochemistry to achieve this separation.

SDS-PAGE involves denaturing proteins by binding them to SDS, a detergent that imparts a negative charge to the proteins, thereby neutralizing their intrinsic charge. This uniform negative charge allows proteins to be separated solely based on their size during electrophoresis. The polyacrylamide gel matrix provides a medium through which proteins migrate based on their molecular weight, with smaller proteins moving faster and farther through the gel than larger ones.

The process begins by preparing the polyacrylamide gel, which is then loaded with the protein sample mixed with a reducing agent to break disulfide bonds. The gel is subjected to an electric field, causing the proteins to migrate through the gel according to their size. Following electrophoresis, the gel is stained to visualize the separated proteins, allowing for analysis and comparison of protein bands.

SDS-PAGE is a powerful tool in biochemistry and molecular biology, enabling researchers to analyze protein samples, determine molecular weights, and assess purity. This technique has revolutionized the field by providing a reliable method for protein separation and characterization, contributing significantly to our understanding of biological processes.

<u>Principle:</u>

The principle of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is based on the separation of proteins according to their molecular weight. The key aspects of this principle are:

1. Protein denaturation: Proteins are denatured by the addition of SDS, a strong anionic detergent, which binds to the proteins and disrupts their native structure. This process ensures that the proteins have a uniform negative charge, independent of their intrinsic charge.
2. Molecular weight-based separation: The denatured proteins are then loaded onto a polyacrylamide gel matrix, which acts as a molecular sieve. During electrophoresis, the proteins migrate through the gel based on their molecular weight, with smaller proteins moving faster and farther than larger ones.
3. Uniform charge-to-mass ratio: The SDS coating ensures that the charge-to-mass ratio of the proteins is approximately constant, allowing for separation based solely on molecular weight, rather than charge.
4. Visualization and analysis: After electrophoresis, the separated protein bands are visualized using staining techniques, such as Coomassie Brilliant Blue or silver staining. This allows for the analysis of protein molecular weights, purity, and relative abundance within the sample.

The combination of these principles enables the effective separation and characterization of complex protein mixtures using SDS-PAGE.

Diagrammatical representation:

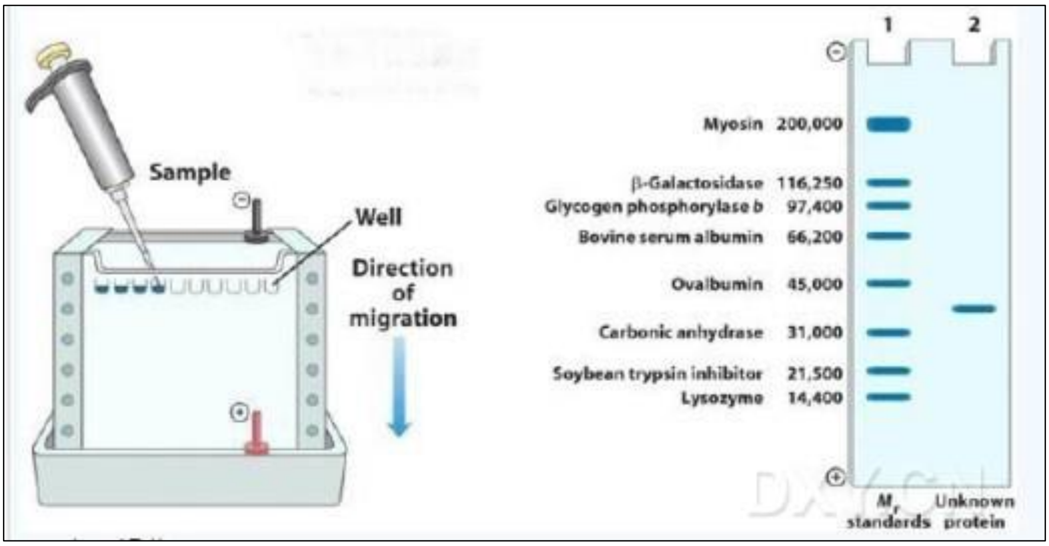

Figure 12: Separation of Protein by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Skills acquired:

Micro-pipetting, gel preparation, gel loading, protein separation, staining & destaining

6. Amplification of DNA using specific primers in Thermal Cycler

Polymerase Chain Reaction (PCR) is a powerful molecular biology technique that allows for the exponential amplification of specific DNA sequences from a small amount of starting material. The process involves the use of a thermal cycler, a specialized instrument that precisely controls the temperature of the reaction mixture to facilitate the various steps of the PCR process.

The key components of a PCR reaction include the DNA template, a pair of short DNA sequences called primers that are complementary to the target DNA region, DNA polymerase enzyme, and the four DNA nucleotides (dNTPs). The thermal cycler is programmed to cycle through three main temperature steps: denaturation, annealing, and extension.

During the denaturation step, the DNA template is heated to a high temperature, typically around 95°C, causing the double-stranded DNA to separate into single strands. In the annealing step, the temperature is lowered, allowing the primers to bind to their complementary sequences on the single-stranded DNA. Finally, in the extension step, the DNA polymerase enzyme synthesizes new DNA strands complementary to the original template, using the bound primers as starting points.

This cycle of denaturation, annealing, and extension is repeated numerous times, typically 20-40 cycles, resulting in an exponential amplification of the target DNA sequence. The amplified DNA can then be visualized and analyzed using various techniques, such as gel electrophoresis or fluorescent dyes.

PCR is widely used in a variety of applications, including genetic analysis, forensics, diagnostics, and research, due to its ability to generate large quantities of specific DNA sequences from small starting samples.

Principle:

The principle of DNA amplification using specific primers in a thermal cycler is based on the Polymerase Chain Reaction (PCR) technique. PCR is a powerful molecular biology tool used to exponentially amplify specific DNA sequences from a small amount of starting material.

The process involves three main steps: denaturation, annealing, and extension. During denaturation, the double-stranded DNA is heated to separate the strands. Then, specific primers designed to target the DNA sequence of interest anneal to their complementary regions on the single-stranded DNA. Finally, a DNA polymerase enzyme extends the primers, synthesizing new complementary DNA strands.

These three steps are repeated in multiple cycles, typically 20-40 times, resulting in the exponential amplification of the target DNA sequence. The thermal cycler precisely controls the temperature and duration of each step, ensuring optimal conditions for the reaction.

The specificity of the amplification is determined by the design of the primers, which are complementary to the target DNA sequence. This allows for the selective amplification of the desired DNA fragment, even from complex samples containing a mixture of genetic material.

Diagrammatical representation:

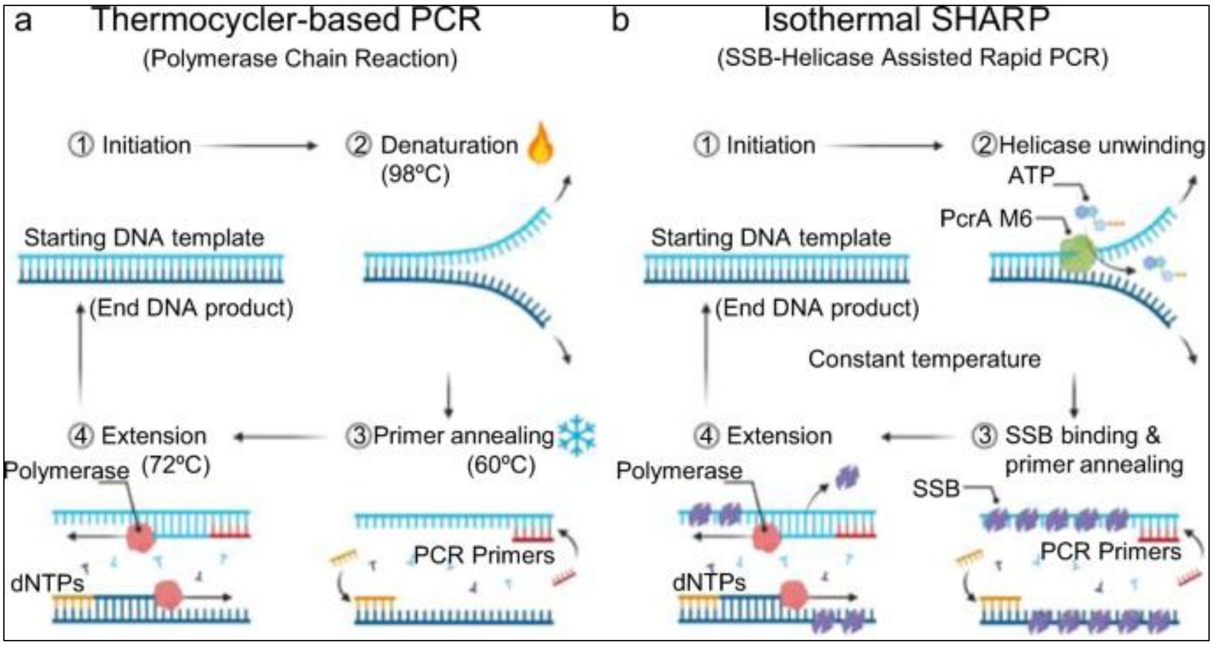

Figure 13: Amplification of DNA using Thermal Cycler

Skills acquired:

Sample preparation, Theoretical knowledge, instrument familiarization and operation of thermal cycler

7. Demonstration of the working of the Flow Cytometry

Flow cytometry is a powerful analytical technique used to measure and analyze the physical and chemical characteristics of cells or particles suspended in a fluid. It is widely used in various fields, including biology, medicine, and immunology, to study cell populations, detect and quantify specific cell types, and analyze cellular processes.

The working of flow cytometry involves the following key steps:

1. Sample preparation: Cells or particles are suspended in a fluid, often a buffer solution, and stained with fluorescent dyes that bind to specific cellular components or molecules of interest.
2. Hydrodynamic focusing: The sample is injected into a flow chamber, where it is surrounded by a sheath fluid. This creates a narrow, single-file stream of cells or particles that pass through a laser beam.
3. Laser excitation and light detection: As the cells or particles pass through the laser beam, the fluorescent dyes are excited, causing them to emit light at specific wavelengths. Detectors positioned around the flow chamber collect this emitted light, which is then converted into electronic signals.
4. Data analysis: The electronic signals are processed and analyzed by a computer, providing information about the size, granularity, and fluorescence intensity of the cells or particles. This data can be used to identify and quantify different cell populations, as well as to study various cellular properties and functions.

Flow cytometry is a versatile technique that has numerous applications, including cell counting, cell sorting, immunophenotyping, and the study of cellular signaling pathways. Its ability to rapidly analyze and sort large numbers of cells makes it an essential tool in many areas of biological and medical research.

Principle:

Flow cytometry operates on the principle of analyzing cells or particles suspended in a fluid by passing them through a laser beam one at a time. The process involves staining the cells with fluorescent dyes that emit light when excited by the laser. As the cells flow through the laser beam, detectors capture the emitted light at specific wavelengths, generating electronic signals. These signals are then processed by a computer to provide information on cell size, granularity, and fluorescence intensity. By analyzing this data, flow cytometry can identify and quantify different cell types within a sample, allowing for the study of cellular characteristics and functions. This technique's ability to rapidly analyze large cell populations and provide detailed information on individual cells makes it invaluable in various fields, including immunology, oncology, and stem cell research.

Diagrammatical representation:

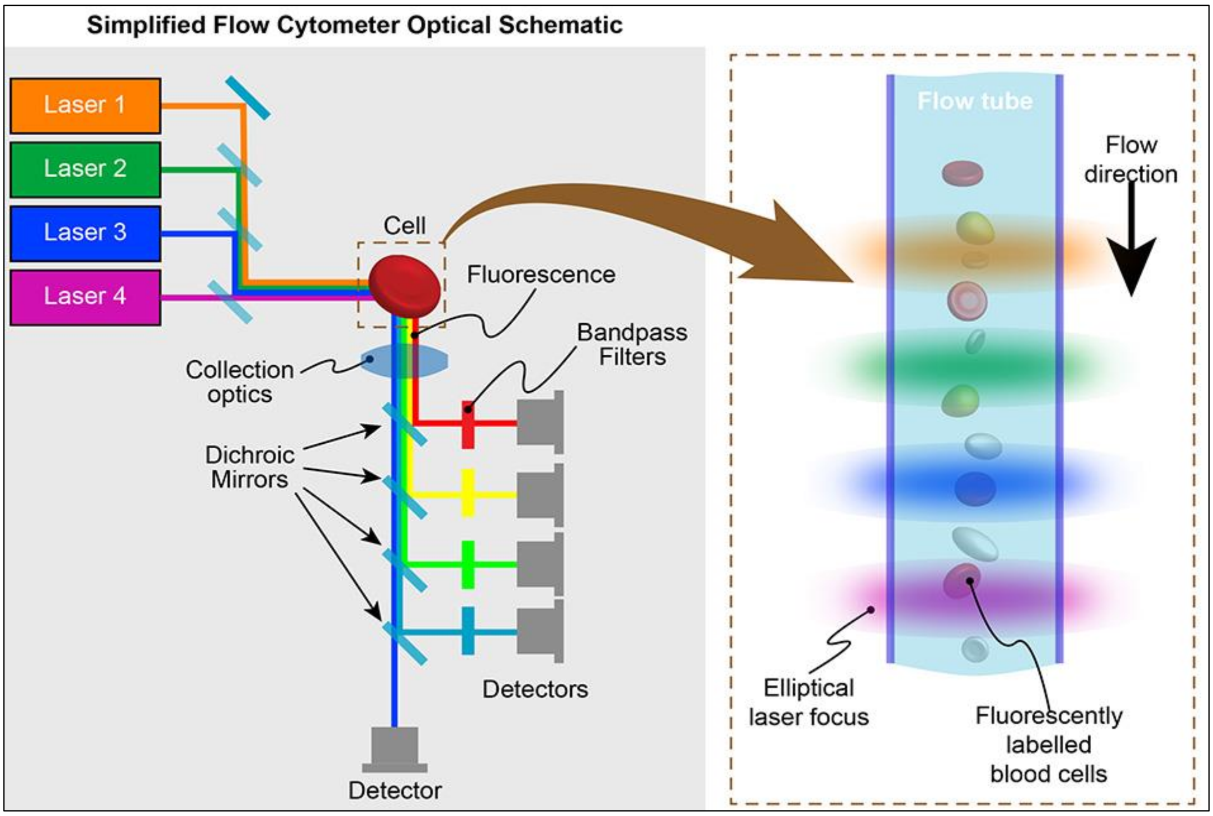

Figure 14: Working of Flow Cytometry

Skills acquired:

Sample preparation, Theoretical knowledge, instrument familiarization and Data interpretation

8. Demonstration of the working of the Real-Time Polymerase Chain Reaction (RT-PCR)

The Real-Time Polymerase Chain Reaction (RT-PCR) is a powerful molecular biology technique that enables the rapid and precise amplification of specific DNA or RNA sequences. This method has become a crucial tool in various fields, including medical diagnostics, genetic research, and forensic analysis. The real-time aspect of this technique allows for the continuous monitoring and quantification of the amplified target sequences during the amplification process, providing valuable insights into the initial quantity of the target molecules.

The RT-PCR process involves several key steps: sample preparation, primer and probe design, thermal cycling, and data analysis. The sample, which may contain the target nucleic acid, is first prepared and extracted. Specific primers and probes are then designed to target the region of interest. The sample is then subjected to a series of temperature cycles, during which the target sequence is repeatedly amplified. The amplification is monitored in real-time through the use of fluorescent dyes or probes, which emit a signal proportional to the amount of amplified product. This data is then analyzed to determine the initial quantity of the target sequence, allowing for accurate quantification and comparison between samples.

The versatility and sensitivity of RT-PCR have made it a widely adopted technique in various applications, from disease diagnosis to gene expression analysis. Its ability to detect and quantify even minute amounts of target molecules has revolutionized the field of molecular biology and continues to drive advancements in scientific research and clinical diagnostics.

Principle:

The principle of Real-Time Polymerase Chain Reaction (RT-PCR) is based on the amplification and detection of a specific DNA or RNA sequence in a sample. The process involves the following key steps:

1. Sample Preparation: The target nucleic acid (DNA or RNA) is extracted and purified from the sample.
2. Primer and Probe Design: Specific primers and probes are designed to target the region of interest within the target sequence.
3. Thermal Cycling: The sample is subjected to a series of temperature cycles, which include denaturation, annealing, and extension steps. This allows for the exponential amplification of the target sequence.
4. Real-Time Detection: During the amplification process, fluorescent dyes or probes are used to monitor the accumulation of the amplified product in real-time. The fluorescent signal is proportional to the amount of amplified product.
5. Data Analysis: The real-time fluorescence data is analyzed to determine the initial quantity of the target sequence, enabling accurate quantification and comparison between samples.

The combination of targeted amplification and real-time detection makes RT-PCR a highly sensitive and specific technique for the detection and quantification of nucleic acid targets.

Diagrammatical representation:

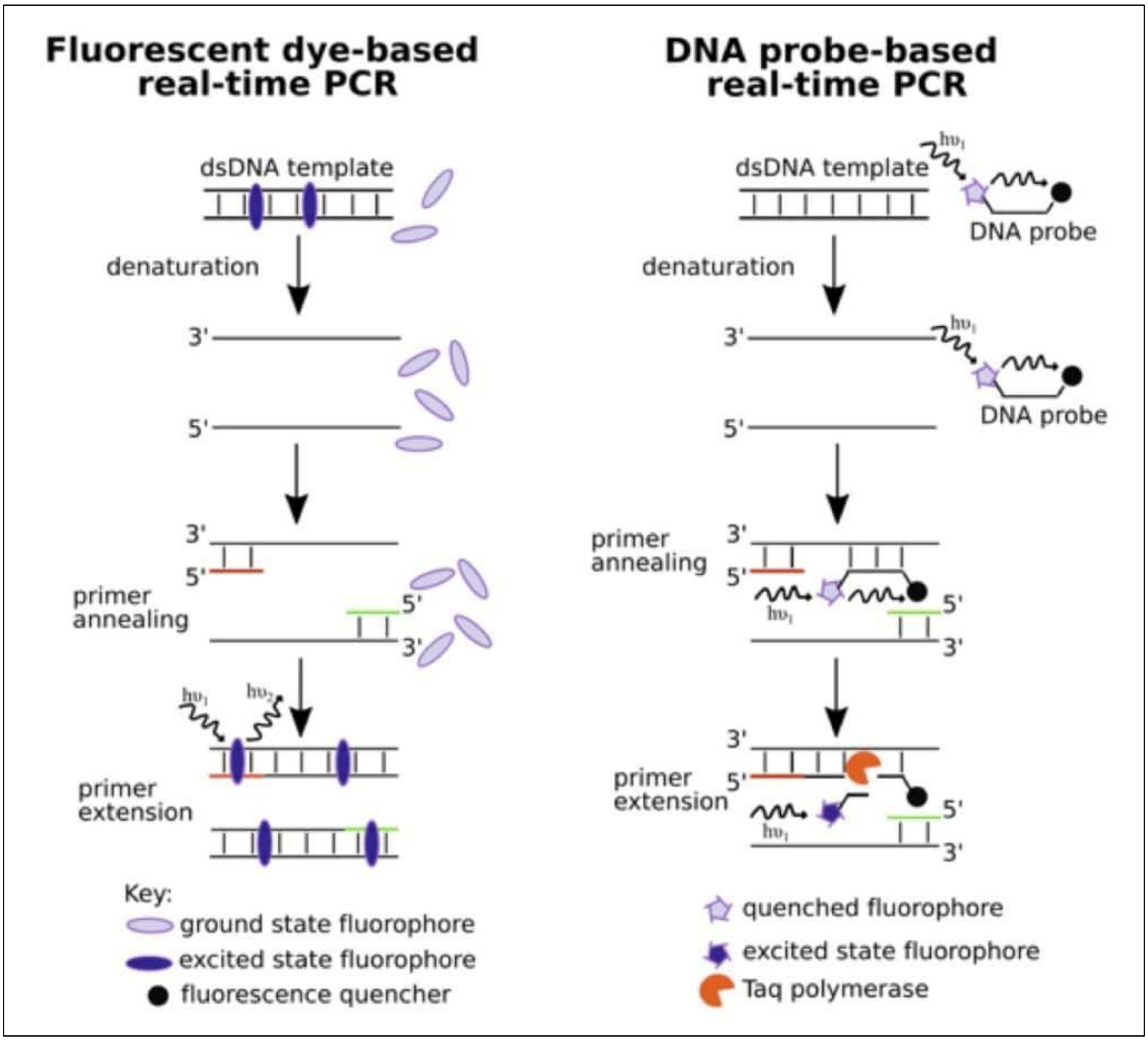

Figure 15: Working of Real-Time PCR (RT-PCR)

Skills acquired:

Sample preparation, Theoretical knowledge, instrument familiarization and importance of individual PCR components and Data interpretation

Conclusion

The hands-on training program at the National Facility for Biopharmaceuticals (NFB) has been an enriching and transformative experience, providing valuable insights and practical skills in molecular biology and proteomic analysis. Throughout the program, participants have gained a deep understanding of various methodologies and techniques essential to the field, laying a strong foundation for future endeavors in biopharmaceutical research and development.

One of the key takeaways from the training program is the comprehensive understanding of molecular biology techniques. Trainees have learned essential skills such as DNA isolation, restriction enzyme digestion, ligation, protein separation, and nucleic acid amplification. These techniques are fundamental to molecular biology research and are widely used in various applications, including gene cloning, DNA sequencing, protein characterization, and diagnostics. By mastering these techniques, participants have acquired the ability to manipulate and analyze DNA, RNA, and proteins, opening up avenues for diverse research opportunities.

Moreover, the training program has highlighted the importance of hands-on experience in scientific education. By actively engaging in laboratory activities, trainees have developed practical skills that cannot be acquired through theoretical knowledge alone. The opportunity to work with state-of-the-art equipment and instrumentation has enhanced their technical proficiency and confidence in conducting experiments. Additionally, interacting with experienced trainers and fellow participants has fostered a collaborative learning environment, where knowledge sharing and peer support have been instrumental in the learning process.

The most valuable aspect of the training experience lies in its contribution to the participants' understanding of the scientific field. By immersing themselves in laboratory work and experimental procedures, trainees have gained firsthand experience of the scientific process—from hypothesis formulation to data analysis. They have learned to critically evaluate experimental results, troubleshoot technical issues, and adapt protocols to achieve desired outcomes. This practical knowledge, combined with theoretical understanding, has equipped participants with a holistic perspective of molecular biology and proteomic analysis, enabling them to approach scientific challenges with confidence and competence.

Furthermore, the training program has broadened participants' horizons by exposing them to cutting-edge technologies and emerging trends in the field. The hands-on experience with Flow Cytometry and Real-Time Polymerase Chain Reaction (RT-PCR), for example, has provided insight into advanced analytical techniques used in biomedical research and clinical diagnostics. By familiarizing themselves with these technologies, participants have expanded their skill set and prepared themselves for future advancements in the scientific field.

Looking ahead, the acquired skills from the training program hold immense potential for various applications and areas of further exploration. Participants are now equipped to pursue research projects in biopharmaceuticals, biotechnology, healthcare, and other related fields. They can contribute to the development of novel therapeutics, diagnostic tools, and biologics, addressing pressing challenges in human health and disease. Additionally, the acquired skills are transferable across industries, opening up opportunities in academia, pharmaceutical companies, research institutions, and biotech startups.

Moreover, the training program has instilled a sense of curiosity and a passion for lifelong learning among participants. As they continue their scientific journey, they are encouraged to explore new avenues of research, collaborate with peers, and stay abreast of advancements in the field. Whether it's delving deeper into specific areas of molecular biology or exploring interdisciplinary intersections with other scientific disciplines, participants are poised to make meaningful contributions to scientific knowledge and innovation.

In conclusion, the hands-on training program at the National Facility for Biopharmaceuticals has been a transformative experience for participants, providing invaluable skills, knowledge, and insights into molecular biology and proteomic analysis. By mastering essential techniques, gaining practical experience, and fostering a collaborative learning environment, participants have been empowered to embark on successful careers in biopharmaceutical research and beyond. As they move forward, they are poised to make significant contributions to scientific advancement, innovation, and the betterment of society as a whole

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